REMARKS

In the Official Action dated June 1, 2006, claims 39, 44-46, 51, 56-58, 60-61, 63-64, 67-68, 86, 94 and 100-104 are pending and under consideration. Claims 39, 44-46, 51, 60, 63-64, 67 and 100-104 are rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Thomson et al. (Science 282: 1145-1147, 1998) in view of Brustle et al. (Science 285: 754-756, 1999). The remaining claims are rejected as allegedly unpatentable over Thomson et al. in view of Brustle et al., and further in view of either or both of Ben-Hur et al. (J. Neurosci. 18: 5777-5788, 1998), and Stemple et al. (Cell 71: 973-985, 1992).

This Response addresses each of the Examiner's rejections. Applicants therefore respectfully submit that the present application is in condition for allowance. Favorable consideration of all pending claims is therefore respectfully requested.

Applicants respectfully submit that, for claimed subject matter to be obvious in view of a combination of prior art references, the prior art (1) must suggest the combination to one of ordinary skill in the art and (2) reveal that one of such skill would have a reasonable expectation of success in carrying out the invention. In re Vaeck, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). As discussed in the following sections, Applicants respectfully submit that the Examiner has failed on both prongs of the two-prong test established by In re Vaeck, and therefore failed to establish a *prima facie* case of obviousness under 35 U.S.C. §103..

Deficiencies in the References and Lack of Suggestion to Combine

(i) The Thomson reference

The Examiner states that the claimed invention is drawn to directed differentiation of hES cells to neural progenitor cells (NPCs) under specific conditions using specific media and

growth factors. The Examiner admits that the Thomson reference did not show that hES cells could differentiate to produce ectodermal cells *in vitro*. However, the Examiner states that the reference teaches that *in vivo*, hES cells form teratomas containing ectodermal cells. Therefore, the Examiner concludes that hES cells have the capacity to form neural cells of the ectodermal lineage given appropriate conditions.

Applicants draw the Examiner's attention to the fact that there has been no *in vitro* demonstration of ectodermal cells being formed that could differentiate and eventually give rise to neural cells. The only conditions disclosed by the Thomson reference, under which hES cells can differentiate into cells of the ectodermal lineage, are *in vivo* conditions. Therefore, Applicants reassert that the Thomson reference does not teach or remotely suggest anywhere any *in vitro* conditions under which hES cells can differentiate into cells of the ectodermal lineage.

To the contrary, the Thomson reference appears to cast doubt with respect to the capacity of hES cells to differentiate, *in vitro*, into cells of the ectodermal lineage. As submitted in the previous response, Thomson teaches differentiation of hES cells *in vitro* into trophoblast and endoderm lineages. Neither of these lineages is relevant to neural cell types, which derive from the ectoderm layer. On the other hand, Thomson demonstrates differentiation of hES cells into derivatives of all three germ layers *in vivo* in the teratoma example. Given the contrast in the Thomson reference between the *in vivo* and *in vitro* differentiation results, those skilled in the art would conclude that it would be difficult to direct differentiation of hES cells towards the neural lineage *in vitro*.

Given the showing in the Thomson reference that *no* ectodermal cells are formed *in vitro*, Applicants respectfully submit that the Thomson reference clearly would not have provided the requisite motivation to those skilled in the art to attempt to obtain NPCs *in vitro*

from hES cells. In fact, the reference arguably could have discouraged those skilled in the art to attempt to obtain NPCs *in vitro* from hES cells.

In this connection, Applicants observe that the Examiner apparently appreciates the differences between *in vivo* and *in vitro* conditions in the context of the claimed invention. Specifically, the Examiner has disputed the relevance of the Xu et al. article, which was referenced in the Colman Declaration to show the differences between mES and hES cells in their differentiation behavior. See page 6, bottom paragraph of the Office Action. The Examiner's opinion is based on the fact that the article relates to *in vivo* studies, whereas the instant claims are directed to *in vitro* differentiation methods. Hence, Applicants respectfully submit that the Examiner's reliance on the Thomson reference is inconsistent with the Examiner's rejection of the Xu reference.

In sum, Applicants respectfully submit that there is no suggestion anywhere in the Thomson reference that would have motivated those skilled in the art to attempt to obtain NPCs *in vitro* from hES cells, much less to obtain NPCs *in vitro* by employing the conditions taught by the Brüstle reference.

(ii) The Brustle reference

Applicants previously submitted that Brustle's teaching is entirely directed to culturing mouse ES cells. As it was evident at the relevant time that the differences between mES cells and hES cells were so substantial, those skilled in the art would not have had a reasonable expectation of success in applying the conditions developed using mES cells, taught by Brustle, to human ES cells in order to obtain NPCs. In addition, Applicants pointed out that the Brustle reference only teaches the derivation of glial precursors which can differentiate to

oligodendrocytes and astrocytes only. The reference fails to teach the production of multipotential NPCs that can give rise to oligodendrocytes, astrocytes and neurons.

The Examiner argues that the presently claimed methods only require the use of a serum free media supplemented with bFGF and EGF in order to produce NPCs, and that these conditions are disclosed in the Brustle reference. According to the Examiner, there are no differences between the culture conditions of the Brustle reference and the claimed methods. Therefore, the Examiner contends that although the reference does not specifically show the formation of neurons under the disclosed conditions, if one were to follow the teachings of the reference, one would *necessarily* reach the same results and produce the same cell types as the presently claimed methods.

In the first instance, Applicants respectfully submit that the instant methods are directed to the production of NPCs from human ES cells, in contrast to the teachings of the reference, which relate to mouse ES cells. The source of cells is certainly a part of the claimed methods, and thus, the Examiner's contention that there are no differences between the reference and the claimed methods fail on this ground alone.

Furthermore, Applicants respectfully submit that the results disclosed in the Brustle reference itself contradict the Examiner's argument. The reference shows that under the culture conditions disclosed therein, which conditions are allegedly the same as those recited in the present claims, mouse ES cells only gave rise to glial precursors and failed to produce any neuronal cells. Applicants question the Examiner's reasoning in this regard – if the conditions disclosed by Brustle et al. were applied by Brustle et al. to mouse ES cells and did not give rise to neuronal cells, how could the Examiner reach the conclusion that the conditions, if applied to

human ES cells, would *necessarily* produce NPCs that give rise to oligodendrocytes, astrocytes and neurons, as presently claimed?

Accordingly, Applicants respectfully submit that the Brustle reference is entirely directed to mouse ES cells. There is no suggestion anywhere in the reference that would have motivated those skilled in the art to apply the conditions disclosed therein to human ES cells in order to obtain multipotential NPCs that give rise to oligodendrocytes, astrocytes and neurons, as presently claimed.

Lack of A Reasonable Expectation of Success

The Examiner concludes that it would have been obvious for one skilled in the art to culture the hES cells of Thomson, under the conditions taught by Brustle, to arrive at the claimed invention, with a reasonable expectation of success.

Applicants respectfully submit that it was evident at the relevant time that the differences between mES cells and hES cells were so substantial that those skilled in the art would not have had a reasonable expectation that hES cells would behave in the same manner as mES cells. Therefore, those skilled in the art would not have had a reasonable expectation of success in producing human NPCs and to further differentiate such human NPCs by simply applying the conditions developed using mES cells, taught by Brustle, to human ES cells.

In Dr. Alan Colman's Declaration provided in the previous Response, the distinctions between mouse ES cells and human ES cells were delineated in Table 1, both in terms of response to chemical cues, culture conditions and cells derived therefrom. The Declaration also referenced several supporting articles that address the distinctions between mES cells and hES cells. Among the referenced articles, Xu et al. (Nature Biotechnology 20: 1261-1264, 2002) reported the induction of differentiation of hES cells to trophoblast using the growth factor

BMP4. In contrast, mES cells cannot form trophoblasts, as discussed in Xu et al. in the bridging paragraph of pages 1262-1263.

In response, the Examiner points out that in discussing the inability of mES cells to form trophoblasts, Xu et al. referred to Beddington et al. as evidence. The Examiner states that the Beddington et al. reference only describes *in vivo* tests of mES cells, and does not provide any comparison of mES cells with hES cells under *in vitro* conditions.

In the first instance, Applicants respectfully submit that the Examiner's position respecting Xu et al. and Beddington et al. is inconsistent with the Examiner's reliance on the teachings of the Thomson reference relating to the *in vivo* behavior of hES cells.

Further, Applicants provide herewith an article by Niwa et al. (Nature Genetics 24: 372-376, 2000) (**Exhibit 1**), which describes that mES cells were converted by genetic engineering into cells capable of making trophoblasts. The need for such a radical approach (i.e., genetic engineering) is consistent with the notion that unlike human ES cells, it is very difficult, if not impossible, to direct mouse ES cells to differentiate to trophoblast cells in culture via a non-genetic engineering approach. Hence, the Niwa reference provides additional evidence showing the differences between mES and hES cells.

As further evidence that mES cells and hES cells behave differently, Applicants provide herewith an article published in *Nature Biotechnology* (18: 399-405, 2000) (**Exhibit 2**). The authors reported that conditions that were used to facilitate formation of embryoid bodies (EBs) from mouse ES cells did not result in formation of EBs from human ES cells. Applicants direct the Examiner's attention to page 401, right column of the article, where it is stated:

> "In these high-density cultures [of human ES cells], there was no consistent pattern of structural organization suggestive of the formation of embryoid bodies similar to those formed in mouse ES cell aggregates or arising sporadically in marmoset ES cell cultures. Cultivation of clumps of ES cells in

hanging-drop cultures, or as aggregates on bacteriological petri dishes, in standard medium without feeder cells resulted in considerable cell death, and only a minority of the clumps survived. There was no evidence of growth or formation of distinct tissue layers in the aggregates. When the surviving clumps were replated onto tissue culture plastic in standard culture medium, cell death was evident, and no extensive outgrowth occurred. Thus, manipulations used in our laboratory and elsewhere to facilitate embryoid body formation and multilineage differentiation of mouse ES cells induced death of human ES cells."

The Examiner, while recognizing that there are different conditions for culturing mES cells and hES cells, states that Applicants have not provided any evidence to "show that hES cells cannot form cells of neuronal lineages due to the gp130 deficiency." Office Action, page 7 (emphasis in original). Additionally, the Examiner again refers to the Thomson reference, which, according to the Examiner, demonstrates the capacity of hES cells to form ectodermal cells given appropriate conditions.

Applicants respectfully submit that the prior art discussed in the Colman declaration was not intended to show that hES cells cannot form cells of neuronal lineages due to the gp130 deficiency. Rather, Applicants intended to establish, and believe to have established, that the prior art at the relevant time cast doubt on the likelihood of success in attempting to derive NPCs from *human* ES cells by applying protocols developed with *mouse* ES cells. Applicants respectfully submit that under In re Vaeck, unobviousness may be established by showing *a lack of a reasonable expectation of success*. Applicants do not need to show that the art at the relevant time indicated to those skilled in the art that it was impossible to arrive at the claimed invention.

As to the Examiner's reliance on the Thomson reference, Applicants reassert that this reference merely shows the potential of hES cells to differentiate into ectodermal cells *in vivo* in the teratoma example. There is no teaching or suggestion in the Thomson reference that human

ES cells would have capacity to differentiate into ectodermal cells *in vitro*. In fact, as discussed above, the *in vitro* results described in the Thomson reference would have suggested to those skilled in the art that it would be difficult to direct differentiation of hES cells towards the neural lineage *in vitro*.

The Examiner states on page 7 of the Office Action that one of skill "would have had a reasonable expectation of success, because hES cells, as mES cells, formed ectodermal cells in a teratoma." Applicants respectfully submit that the Examiner's reasoning is flawed and inconsistent with the legal standard set forth in In re Vaeck. The relevant inquiry is whether those skilled in the art would have had a reasonable expectation of success in arriving at *the claimed invention*. Here, the claimed invention is the production of NPCs *in vitro*, not *in vivo* in a tertoma. Neither the Thomson reference nor the Brustle reference has provided those skilled in the art with a reasonable expectation of success in producing human NPCs *in vitro* by applying the conditions developed with mouse ES cells, taught by Brustle, to human ES cells. That is, the references simply did not provide those skilled in the art with a reasonable expectation of success in arriving at *the claimed invention*.

Applicants further respectfully submit that at the priority date of the present application, human ES cell research was still in its infancy, and conditions for culturing and differentiation were more established for mouse ES cells as compared to human ES cells. The art at the time reflects the uncertainties and difficulties associated with deriving differentiated cells from hES cells. For example, U.S. Patent No. 6, 602, 711 to Thomson, which has a filing date of February 21, 2000 (only 3 weeks before the priority date of the present application), describes the state of the art at the time as follows:

> "[H]uman and mouse embryos differ in the timing of the embryonic gene expression, in the formation, structure and function of the fetal membranes

and placenta and in the formation of an embryonic disc instead of an egg cylinder ....." (Fourth paragraph in the Background section.)

"Analogous experiments on primate ES cells demonstrated that embryoid body formation by conventional murine protocols fail.....We have learned that primate ES cells die rapidly when dispersed to single cells if attachment is prevented ....." (Penultimate paragraph in the Background section).

"[A] need exists for improved methods for producing primate EB, and differentiated cells therefrom". (Last paragraph of the Background section.)

Therefore, the '711 patent further supports the notion that at the priority date of the present application, those skilled in the art understood that hES and mES were different such that methodologies developed with mouse ES cells could not be easily extrapolated to human ES cells.

Furthermore, the '711 patent suggests deriving differentiated cells from hES cells based on a method involving the formation of EB. Similarly, the differentiation approach for mouse ES cells, taught by Brustle, also involved the formation of EB. In fact, at the priority date of the present application, EB formation was generally thought to be crucial to any subsequent differentiation. In contrast, according to the methodology of the present application, EB formation is, surprisingly, not necessary for producing differentiated cells from hES cells. Therefore, Applicants respectfully submit that those skilled in the art would not have had a reasonable expectation of success in attempting to differentiate hES cells without first forming EBs.

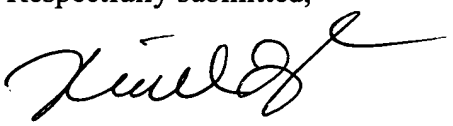
To summarize, Applicants respectfully submit that neither Thomson nor Brustle would have provided a motivation to combine the respective teachings and apply the conditions taught by Brustle to human ES cells in order to produce human NPCs. Further, even assuming, *pro arguendo*, some motivation was provided, those skilled in the art would not have had a

reasonable expectation of success in applying the conditions taught by Brustle to human ES cells to produce human NPCs, much less to produce human NPCs having the multipotent differentiation capacity, as presently claimed.

Therefore, Applicants respectfully submit that all the obviousness rejections, which rely on the combination of Thomson and Brustle, are improper. Withdrawal of the rejections is respectfully requested.

In view of the foregoing amendments and remarks, it is firmly believed that the subject application is in condition for allowance, which action is earnestly solicited.

Respectfully submitted,



Xiaochun Zhu
Registration No. 56,311

SCULLY, SCOTT, MURPHY & PRESSER, P.C.
400 Garden City Plaza, Suite 300
Garden City, New York 11530
(516) 742-4343
XZ:ab

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